

Chemical prey cues influence the urban microhabitat preferences of Western black widow spiders, *Latrodectus hesperus*

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Abstract. Spiders are important predators in terrestrial ecosystems, and several spider species have been shown to use chemical cues to locate prey. However, the extent to which chemical prey cues actually drive habitat use by individual spiders remains unclear. In this study we tested whether Western black widow spiders, *Latrodectus hesperus* Chamberlin & Ivie 1935, can detect chemical cues left by potential prey items and adjust their habitat preferences (i.e., web building behavior and refuge choice) accordingly. Using outdoor enclosures, we gave mature female widows the choice of microhabitat (rocks) previously housing cricket prey versus control rocks lacking cricket prey. Our results showed a significant preference by black widows to build their webs in areas that contain chemical prey cues. We discuss the implications of this finding for our understanding of urban black widow habitat use, population dynamics, and the potential for urban infestations.

Keywords: Foraging kairomone, urban ecology, web building, predation

Despite being the oldest method of animal communication, olfaction and chemical communication have historically been less studied than visual and acoustic communication (Bradbury & Vehrencamp 1998). However, development of insect model systems has allowed for great advances in the field of chemical ecology (reviewed in Symonds & Elgar 2008). For example, insect models have helped identify the widespread prevalence of sexual pheromones that bring males and females together, sometimes from great distances, and initiate the mating sequence (Cardé & Minks 1995). In addition, predator-prey studies involving arthropods have been instrumental in the rapidly growing study of kairomones, chemical cues emitted by a sender that are exploited by a receiver (Dicke & Sabelis 1988; Dicke & Grostal 2001; see Ruther et al. 2007 for expanded definitions of kairomones). In particular, foraging kairomones allow predators to locate prey (e.g. Clark et al. 2000; Punzo 2006), and enemy-avoidance kairomones allow prey to avoid predators (Kortet & Hedrick 2004). Thus, chemical signals are a critical factor shaping the evolution of arthropod mating systems and predator-prey dynamics.

Despite a historical emphasis on web-based, vibratory communication, spiders have proven effective model species for the study of pheromonal communication (reviewed in Gaskett 2007; Schulz et al. 2004). In contrast, the study of chemical cues mediating predator-prey dynamics involving spiders has received less attention, despite the widespread belief that spiders are key predators of terrestrial ecosystems (Wise 1993). One system that has received considerable attention in this respect involves the wolf spider *Hogna helluo* (Walckenaer 1837) and its syntopic prey species, the smaller wolf spider, *Pardosa milvina* (Hentz 1844). Wolf spiders (Lycosidae) are a wandering, non web-building taxon found in great abundances in agricultural ecosystems of the United States (Marshall & Rypstra 1999). From the perspective of predation risk, *P. milvina* exhibit anti-predator behavioral responses to airborne (Schonewolf et al. 2006) as well as silk

and excreta-based, chemical cues from *H. helluo* (Persons et al. 2001, 2002; Lehmann et al. 2004). Conversely, from the perspective of prey availability, *H. helluo* recently fed *P. milvina* prefer areas laden with chemical cues from *P. milvina*, whereas *H. helluo* recently fed crickets prefer areas laden with chemical cues from crickets (Persons & Rypstra 2006). A similar preference for patches laden with cricket chemical cues has been shown for the wolf spider, *Schizocosa ocreata* (Persons & Uetz 1996). Thus, chemical cues from prey play an important role in shaping the foraging behavior of wolf spiders.

Given that spiders are predators thought to limit insect populations, it is surprising that more studies have not tested the ability of non-lycosid spiders to detect chemical cues from their prey (but see Suter et al. 1989; Allan et al. 1996; Clark et al. 2000 for a few examples). Particularly lacking are field/mesocosm studies of kairomone use by web-building spiders that examine spider habitat/web building preferences outside of the laboratory. Web-building spiders make a critical microhabitat selection decision when they invest the time and energy required to build and maintain a web. Optimal web location is likely influenced by food availability, predation risk, and competition (Smallwood 1993), as well as other factors (e.g. body condition, see reviews by Janetos 1986; Riechert & Gillespie 1986; Herberstein & Tso 2011). Thus, selection might favor web placement in areas of prey abundance, and chemical cues from prey are one cue of prey availability/abundance.

Here we test the idea that a locally abundant web-building spider, the Western black widow (*Latrodectus hesperus* Chamberlin & Ivie 1935), uses chemical prey cues to determine where to place its web. Widow spiders (*Latrodectus* spp.) are perhaps best known for the potency of their venom (Orlova et al., 2000) and the medical concern these toxins present to human victims (Muller 1993; Gonzalez 2001). Moreover, widow spiders have proven to be outstanding urban, agricultural and invasive pests (Costello & Daane 1999; Daane et al. 2004; Garb et al. 2004). In particular, *L. hesperus* populations in urban habitats of Phoenix, Arizona (e.g.,

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schoolyards) can reach 0.28 spiders/m², with reduced nearest neighbor distances (mean = 1.9 m) relative to populations from surrounding, undisturbed Sonoran desert habitat (density = 0.006 spiders/m² and nearest neighbor distance > 50 m; Johnson unpub. data). *Latrodectus hesperus* males use silken cues from females in a courtship context (Ross & Smith 1979; Johnson et al. 2011), and widow web architecture is condition dependent, with spiders adaptively allotting more to sticky, prey-capture silk when prey are limiting (Blackledge & Zevenbergen 2007). Here we present the first study to ask whether or not this common urban pest bases its microhabitat preferences on chemical cues from prey. Specifically, we utilize an outdoor enclosure that mimics urban habitat and test the prediction that female *L. hesperus* will preferentially choose habitat patches that have recently housed cricket prey for their webs.

METHODS

Housing.—We used an outdoor facility on the grounds of Arizona State University's West campus approximately 7 m² in area and 165 cm tall, contained on the sides by chain link fencing and with a chicken wire ceiling. Within this facility, a metal wall (0.75 m tall) was buried into the ground to create a 6-m² enclosure. Within this enclosure, we buried aluminum flashing into the soil to divide the enclosure into 16 cells of equal area (25 cm tall, 1.5 m²) to maintain individual spiders/webs.

Within each of the 16 cells, one commercially-purchased river rock (average diam. = 25.5 cm) was placed in each of the four corners. To minimize pre-experiment chemical cues that rocks may have been exposed to before purchase, we submerged all rocks in water for 48 h, scrubbed rock surfaces with a metal pad and rinsed rocks with water before use. Thus, non-experimental cues were minimized, and all rocks used were treated in the above manner before being used. Rocks were used in only one trial.

Establishing prey chemical cues.—One rock/corner from each of the 16 cells was randomly selected to be charged with cricket chemical cues (hereafter referred to as 'prey-cue corners'). In order to "charge" the rock with the prey chemical cues, we housed five adult house crickets (*Acheta domesticus*) for five continuous days under a plastic, translucent tub (48 cm × 308 cm × 14 cm LWH), upturned to contain the experimental rock. Prey-cue corners were inspected daily, and dead crickets were removed and replaced. After five days, plastic tubs and crickets were removed. Finally, prey-cue corners were finished by the addition of egg crate (10 × 10 × 4 cm LWH) that crickets had been shipped in to provide additional chemical cues. This same size of egg crate, purchased from a local grocery store (i.e., housing chicken eggs but not arthropods), was added to each of the non prey-cue corners.

Experimental procedure.—We collected 48 penultimate-stage juvenile females from urban Phoenix habitats in April 2009. Spiders were housed individually in the laboratory in plastic, transparent containers (10 × 10 × 13 cm LWH) and fed one adult house cricket weekly until trials began. Spiders matured in the laboratory within two to three weeks of collection and were used in trials within the first four weeks of maturity. Spiders were weighed (mg) and had their cephalothorax digitally imaged immediately prior to use. We estimated fixed adult body size from this single image as the length (mm) of the tibia from a fourth leg using Zoombrowser™. We

calculated spider body condition as both the residuals of a linear regression of body mass on tibia length (Jakob et al. 1996), as well as body mass corrected for tibia length (Moya-Larano et al. 2008). As these condition measures yielded similar results, we used mass corrected for tibia length in the analyses reported.

Trials were conducted in May 2009, when average daily temperatures in Phoenix ranged from lows of ~ 12° C to highs of ~ 30° C. At 1900 hours, spiders were randomly chosen and released in the center of each cell. We recorded initial direction of movement and then scored the location of each spider every 15 min for 90 min. Any spiders not located during a check were assumed to be hiding within the cell (under rocks or egg crate) and allowed to remain hiding for three consecutive intervals. On the fourth "hiding" interval, egg crates and rocks were gently maneuvered to determine the location of the spider. Spiders were assumed to have been in this location for the entirety of the "hiding" interval. In the cases in which a spider was found moving within its cell, we scored the spider's location as the corner toward which the spider moved. The following day we scored each spider's location again at 0900 and 1800 h. On the third day after introduction, we made a final 0900 h observation, and considered this to be each spider's final habitat selection. We used this third morning location as our measure of habitat selection, because no spider relocations occurred for five days after this date, and all spiders not missing ($n = 32$ spiders out of 48 released, see below) had begun to build extensive webs out from the rock they were using as a daytime refuge.

Statistical analysis.—We conducted three block replicates of this experiment - each using an independent sample of spiders and rocks. The aluminum sides and dirt floor of each cell was sprayed with water through a high pressure hose and allowed to air-dry over the course of at least seven days before the next block replicate began. The location of the prey-cue corner in a cell was randomly selected before each trial. Thus, although we cannot claim to have removed all residual chemical cues from crickets and previous spiders, our aim was to minimize these previous cues so as not to interfere with our manipulation of prey-cue corners. We view the evidence below that habitat preferences did not differ between our three block replicates as evidence that we succeeded in minimizing the accumulation of cues across blocks.

In block 1, 12/16 spiders were accounted for after three days. In block 2, 13/16 spiders were accounted for after three days. In block 3, 7/16 spiders were accounted for after three days. Thus, across three blocks of the experiment 32 spiders established webs (see below for a discussion of the fate of the 16 missing spiders). Missing spiders were excluded from habitat preference analyses. All analyses were conducted using SPSS. For each block replicate the observed frequency of habitat choice of prey-cue corners by spiders (at Day 3) was compared to that expected by random chance (25%) and analyzed with *G*-tests. In addition, binary logistic regression was used to test the hypothesis that female condition explained habitat choice (choice of prey cue corner or choice of non prey-cue corner), and multinomial logistic regression was used to examine habitat choosiness (i.e., the number of habitat switches exhibited across the course of the three-day trial; range 0-3).

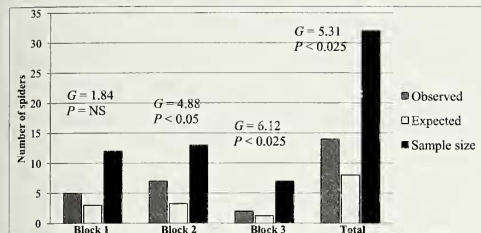


Figure 1.—The number of spiders observed to select prey-cue corners was consistently greater than that expected based on chance. Three consecutive blocks of the experiment are presented, followed by a summation of all three blocks.

RESULTS

Sixty-seven percent (32/48) of the females settled successfully within their cell after three days (i.e., did not escape or get preyed upon; see Discussion). As the habitat choice preferences of missing spiders cannot be known, below we exclude the 16 data points for missing females. All the females not missing settled under one of the four rocks provided as refuge and built their webs out from this substrate. Because rocks were the only refuge from which webs were built, and given that each cell was provisioned with four, evenly spaced rocks, female habitat choice consisted of one of these four options. Black widow females were significantly more likely to select prey-cue corners/rocks for their webs (14/32 = 44%) than would be expected by chance (25% * 32 = 8; $G = 5.29$, $P < 0.05$). As seen in Fig. 1, spider preference for prey-cue corners fluctuated in intensity across the three waves of the experiment, though females were always observed to choose prey-cue corners more often than expected by chance. In addition, using a single heterogeneity G -test (Sokal & Rohlf 1995:715), we found no significant differences in habitat choice across these three repeated blocks of the experiment ($G = 1.24$, $0.25 < P < 0.5$) and thus feel justified in pooling data across blocks.

Female body condition did not predict habitat choice. Binary logistic regression indicated that female body condition (mass corrected for leg length) was a poor predictor of both 1) the original 'choice' to settle under a rock ($n = 48$, $\chi^2 = 0.765$, $P = 0.382$), and 2) the subsequent 'choice' by spiders that settled on a rock to settle on rocks charged with prey cues (266.90 ± 28.5 mg) or not (279.1 ± 20.5 mg; $n = 32$, $\chi^2 = 1.50$, $P = 0.220$). In addition, linear regression indicated that body condition was a poor predictor of the number of habitat switches ("choosiness"), range 0–3 exhibited over the three-day trial ($R^2 = 0.06$, $F_{1,30} = 0.86$, $P = 0.37$), and multinomial logistic regression suggests that this number of switches was a poor predictor of whether the spider eventually chose a prey-cue corner or non-prey-cue corner or was missing ($n = 48$, $\chi^2 = 5.68$, $P = 0.46$). Thus, habitat choice seems to be more strongly shaped by prey cues than by body condition, and we found no suggestion that a spider's ultimate (Day 3) habitat choice was affected by the number of times a spider changed its habitat (choosiness).

DISCUSSION

Our results indicate that urban black widow females preferentially settle in microhabitats/refuges that have been

charged with chemical cues from cricket prey. Thus, although *Latrodectus* spp. are known to use chemical cues from conspecific silk in courtship and cannibalism contexts (Stoltz et al. 2007; Johnson et al. 2011), *L. hesperus* females are also capable of detecting chemical cues from potential hetero-specific prey items. However, we found no evidence that female body condition affected habitat choice for prey availability. It should be noted, however, that we did not manipulate body condition, and thus it remains to be seen whether extreme variation in body condition might intensify habitat-choice decisions for prey cues. Nevertheless, the present evidence that black widows settle near cricket chemical cues adds to the list of spiders known to use foraging kairomones and has important implications for the control of urban infestations.

Our finding that widow spiders use chemical prey cues in their habitat choice has potential implications for the management of urban black widow infestations. The medical importance of this species combined with its ability to form dense urban infestations has led to heightened pesticide usage, and therefore we suggest it is critical that we understand what cues from urban habitat are being used by widow spiders to determine their habitat preferences. If urban widow infestations track the abundance of urban arthropod prey, then the pesticide applications that often follow widow infestations may be effective in controlling widow abundance indirectly by controlling their prey. Although pesticides broadly applied are generally ineffective at directly killing black widows (J.C. Johnson pers. obs.), prey elimination may limit urban widow infestations.

However, such strategies may have unintended negative consequences. Specifically, very little is known about the ability of arthropod pests to behaviorally avoid pesticides. If pesticides are broadly applied, for example, along the perimeter of a schoolyard, the result could be the dispersal of crickets into surrounding areas rather than cricket mortality. Indeed, few data are available to assess the degree to which widespread pesticide applications actually kill urban arthropods, and studies on non-target species indicate that insecticide irritability (avoidance after contact) and insecticide repellence (avoidance without contact) occur frequently (Cordeiro et al. 2010). Anecdotally, our laboratory (one of the few to ban University-sponsored pesticide applications) experiences this behavioral avoidance of pesticides and is overrun by roaches soon after surrounding laboratories are treated. If urban prey are driven away from pesticides instead of killed, then cricket abundance may be lessened in pesticide-treated areas, but will be heightened in surrounding areas—thereby encouraging black widow spiders to relocate. The resultant cycle (i.e., widow infestation, pesticide application, prey relocation, widow infestation) seems a dubious pest control strategy given the costs (financial and environmental health) of wide-scale pesticide usage. We are currently investigating the effects of multiple stressors (pesticides and predation risk) on both urban black widows and their prey.

During this study, we "lost" 1/3 of the spiders released. Although two of these spiders were documented as cannibalized in a neighboring cell, we could not account for the remaining 14 spiders. Despite the fact that the walls that surround our enclosure are 0.75 m. tall and made of slick

aluminum, it is possible that spiders climbed these walls and escaped, though we have never witnessed nor found draglines indicating an escape. In our opinion, it is more likely that the majority of these missing spiders were preyed upon within their cells by vertebrate predators, a possibility that is supported by our finding spider legs in vacated cells. Supporting this hypothesis, we regularly found the native tree lizard *Urosaurus ornatus* as well as the exotic Mediterranean gecko *Hemidactylus turcicus* within spider cells despite our attempts to exclude these predators from the area. Although we can find no report of these species feeding upon *L. hesperus*, we have since verified that when confined with black widows, both species do kill and consume large female widow spiders with no apparent negative effects. We suggest that urban lizards/geckos, widow spiders and crickets offer an outstanding tri-trophic interaction upon which to base the development of an urban food web for the Phoenix metropolitan area.

LITERATURE CITED

- Allan, R.A., M.A. Elgar & R.J. Capon. 1996. Exploitation of an ant chemical alarm signal by the zodariid spider *Habronestes bradleyi* Walckenaer. Proceedings of the Royal Society of London B 263:69-73.
- Blackledge, T.A. & J.M. Zevenbergen. 2007. Condition-dependent spider web architecture in the western black widow, *Latrodectus hesperus*. Animal Behaviour 73:855-864.
- Bradbury, J.W. & S.L. Vehrencamp. 1998. Principles of Animal Communication. Sinauer, Sunderland, Massachusetts.
- Cardé, R.T. & A.K. Minks. 1995. Control of moth pests by mating disruption-successes and constraints. Annual Review of Entomology 40:559-585.
- Clark, R.J., R.R. Jackson & B. Cutler. 2000. Chemical cues from ants influence predatory behavior in *Habrocestum pulex*, an anti-eating jumping spider (Araneae, Salticidae). Journal of Arachnology 28: 309-318.
- Cordeiro, E.M.G., A.S. Correa, M. Venzon & R.N.C. Guedes. 2010. Insecticide survival and behavioral avoidance in the lacewings *Chrysoperla externa* and *Ceraeochrysa cubana*. Chemosphere 81: 1352-1357.
- Costello, M.J. & K. Daane. 1999. Abundance of spiders and insect predators on grapes in Central California. Journal of Arachnology 27:531-538.
- Daane, K.M., P. Hernandez, G.Y. Yokota, W.J. Bentley & A.B. Lawson. 2004. Control tactics and field biology for black widow spiders in table grapes. In 2003-2004. Viticulture Research Report (California Table Grape Commission Annual Report), Volume 32.
- Dicke, M. & P. Grostal. 2001. Chemical detection of natural enemies by arthropods: an ecological perspective. Annual Review of Ecology and Systematics 32:1-23.
- Dicke, M. & M.W. Sabelis. 1988. Infochemical terminology: based on cost-benefit analysis rather than origin of compounds. Functional Ecology 2:131-139.
- Garb, J.E., R.G. Gillespie & A. Gonzalez. 2004. The black widow spider genus *Latrodectus* (Araneae, Theridiidae): phylogeny, biogeography, and invasion history. Molecular Phylogenetics and Evolution 31:1127-1142.
- Gaskett, A. 2007. Spider sex pheromones: emission, reception, structures, and functions. Biological Reviews of the Cambridge Philosophical Society 82:27-48.
- Gonzalez, F. 2001. Black widow bites in children. Journal of the American Osteopathic Association 101:229-231.
- Herberstein, M. & I. Tso. 2011. Spider webs: evolution, plasticity and diversity. Pp. 57-98. In Spider Behavior: Flexibility and Versatility. (M. Herberstein, ed.). Cambridge University Press, Cambridge, UK.
- Jakob, E.M., S.D. Marshall & G.W. Uetz. 1996. Estimating fitness: a comparison of body condition indices. Oikos 77:61-67.
- Janetos, A.C. 1986. Web-site selection: are we asking the right questions? Pp. 9-22. In Spiders: Webs, Behavior, and Evolution. (W.A. Shear, ed.). Stanford University Press, Stanford, California.
- Johnson, J.C., P. Trubl, V. Blackmore & L. Miles. 2011. Male black widows court well-fed females more than starved females: silken cues indicate sexual cannibalism risk. Animal Behaviour 82: 383-390.
- Kortet, R. & A. Hedrick. 2004. Detection of the spider predator, *Holocna nedra*, by naive juvenile field crickets (*Gryllus integer*) using indirect cues. Behaviour 141:1189-1196.
- Lehmann, L.M., S.E. Walker & M.H. Persons. 2004. The influence of predator sex on chemically mediated antipredator response in the wolf spider *Pardosa milvina* (Araneae, Lycosidae) Ethology 110: 323-339.
- Marshall, S.D. & A.L. Rypstra. 1999. Patterns in the distribution of two wolf spiders (Araneae, Lycosidae) in two soybean agroecosystems. Environmental Entomology 28:1052-1059.
- Moya-Larano, J., R. Macias-Ordóñez, W.U. Blanckenhorn & C. Fernandez-Montraveta. 2008. Analyzing body condition: mass, volume or density? Journal of Animal Ecology 77:1099-1108.
- Muller, G.J. 1993. Black and brown widow spider bites in South Africa—a series of 45 cases. South African Medical Journal 83: 399-405.
- Orlova, E.V., M.A. Rahman, B. Gowen, K.E. Volynski, A.C. Ashton, C. Maanser, M. Van Heel & Y.A. Ushkaryov. 2000. Structure of alpha-latrotoxin oligomers reveals that divalent cation-dependent tetramers form membrane pores. Nature Structural & Molecular Biology 7:48-53.
- Persons, M.H. & A.L. Rypstra. 2000. Preference for chemical cues associated with recent prey in the wolf spider *Hogna helluo* (Araneae, Lycosidae). Ethology 106:27-35.
- Persons, M.H. & G.W. Uetz. 1996. Wolf spiders vary patch residence time in the presence of chemical cues from prey (Araneae, Lycosidae). Journal of Arachnology 24:76-79.
- Persons, M.H., S.E. Walker & A.L. Rypstra. 2001. Wolf spider predator avoidance tactics and survival in the presence of diet-associated predator cues (Araneae, Lycosidae). Animal Behaviour 61:43-51.
- Persons, M.H., S.E. Walker & A.L. Rypstra. 2002. Fitness costs and benefits of antipredator behavior mediated by chemotactile cues in the wolf spider *Pardosa milvina* (Araneae, Lycosidae). Behavioral Ecology 13:386-392.
- Punzo, F. 2006. Female spider wasps, *Anophis splendens* Driesbach (Hymenoptera, Pompilidae), learn to associate the odor of host feces with the presence of the host. Journal of Entomological Science 41:202-210.
- Riechert, S.E. & R.G. Gillespie. 1986. Habitat choice and utilization in web building spiders. Pp. 23-48. In Spiders: Webs, Behavior, and Evolution. (W.A. Shear, ed.). Stanford University Press, Stanford, California.
- Ross, K. & R.L. Smith. 1979. Aspects of the courtship behavior of the black widow spider, *Latrodectus hesperus* (Araneae, Theridiidae), with evidence for the existence of a contact sex pheromone. Journal of Arachnology 7:69-77.
- Ruther, J., L.M. Stahl, S. Steiner, L.A. Garbe & T. Tolasch. 2007. A male sex pheromone in a parasitic wasp and control of the behavioral response by the female's mating status. Journal of Experimental Biology 210:2163-2169.
- Schonewolf, K.W., R. Bell & A.L. Rypstra. 2006. Field evidence of an airborne enemy-avoidance kairomone in wolf spiders. Journal of Chemical Ecology 32:1565-1576.
- Schulz, S., J. Fuhendorff, J.L.M. Steidle, J. Collatz & J.T. Franz. 2004. Identification and biosynthesis of an aggregation pheromone of the storage mite *Chortoglyphus arcuatus*. ChemBioChem 5: 1500-1507.

- Smallwood, P.D. 1993. Web-site tenure in the long-jawed spider - Is it risk-sensitive foraging, or conspecific interactions. *Ecology* 74: 1826–1835.
- Sokal, R.R. & F.J. Rohlf. 1995. *Biometry*. Third edition. W.H. Freeman, New York.
- Stoltz, J.A., J.N. McNeil & M.C.B. Andrade. 2007. Males assess chemical signals to discriminate just-mated females from virgins in redback spiders. *Animal Behaviour* 74:1669–1674.
- Suter, R.B., M. Shane & A.J. Hirscheimer. 1989. Spider vs. spider - *Frontinella pyramitela* detects *Argyrodes trigonum* via cuticular chemicals. *Journal of Arachnology* 17:237–240.
- Symonds, M.R.E. & M.A. Elgar. 2008. The evolution of pheromone diversity. *Trends in Ecology & Evolution* 23:220–228.
- Wise, D.H. 1993. *Spiders in Ecological Webs*. Cambridge University Press, Cambridge, UK.

Manuscript received 22 February 2011, revised 11 September 2011.